8. PHYTOPLANKTON AND MICROBIAL PLANKTON OF THE BAY OF BISCAY AND WESTERN IBERIAN SHELF

Xosé Anxelu G. Morán, Ana B. Barbosa, Marta Revilla, Antonio Bode, Emma Orive, and Dominique Soudant

This region of the Northeast Atlantic represents a gradient between two oceanographic regimes: (i) the subtropical waters of southern Portugal and southwestern Spain, and (ii) the temperate waters characterizing the northwestern and northern Iberian coasts (Cantabrian Sea) and the rest of the Bay of Biscay along the French coast. As a consequence, seasonal patterns in physico-chemical and biological variables typical of temperate pelagic ecosystems tend to be more conspicuous as we move northwards and into the Bay. Overall, a strong thermal stratification around summer is followed by vigorous winter mixing, frequently to the bottom of the continental shelf (Lavin *et al.*, 2006). Freshwater outflow from numerous rivers and coastal lagoons contributes to the existence of buoyant low-salinity plumes, particularly during late winter and spring, along the Bay of Biscay (Puillat *et al.*, 2004) and the western (western Iberian Plume) and southwestern Iberian coasts (Lafuente and Ruiz, 2007; Relvas *et al.*, 2007).

The western Iberian Peninsula and the southern Bay of Biscay continental shelves lie at the northernmost reaches of the northwest African upwelling system (Arístegui *et al.*, 2006). Upwelling and downwelling favourable periods, seemingly linked to the North Atlantic Oscillation (NAO) index, are variable in terms of duration, intensity, and phasing, but generally occur during spring–summer and autumn–winter, respectively (Wooster *et al.*, 1976; Alvarez *et al.*, 2008). Upwelling-producing winds vary according to coastline orientation, ranging from northerly winds along the western Iberian coast, easterly winds in the southern Bay of Biscay, and westerly winds in southwestern Iberia. Along the Cantabrian Shelf, the intensity and frequency of upwelling events decrease eastward as temperature and stratification increase (Lavin *et al.*, 2006). The whole region is affected by a saline, warm-slope current flowing poleward during autumn and winter, the Portugal Coastal Counter Current (PCCC; Álvarez-Salgado *et al.*, 2003) also known as the Iberian Poleward Current (IPC). Similar to upwelling episodes, the influence of the IPC along the southern Bay of Biscay decreases eastwards (Pingree and Le Cann, 1990). Other important hydrographic features include: (i) the presence of slope-water anticyclonic eddies in offshore waters of the Bay of Biscay (Pingree and Le Cann, 1993), which affect planktonic assemblages (Fernández *et al.*, 2004); (ii) a persistent frontal structure between Eastern North Atlantic Central Water (ENACW) of subtropical and subpolar origin at the subsurface off Cape Fisterra (Arístegui *et al.*, 2006); and (iii) other mesoscale features such as upwelling filaments, fronts, and eddies off southwest Iberia (Lafuente and Ruiz, 2007; Relvas *et al.*, 2007). Overall, the

Figure 8.1
Locations of the Bay of Biscay and western Iberian Shelf plankton monitoring areas (Sites 46–54) plotted on a map of average chlorophyll concentration.

region comprises extreme variability with respect to coastal configuration, shelf width, coastal upwelling intensity, riverine outflow, mesoscale activity, and retentive vs. dispersive physical mechanisms (see Arístegui *et al.*, 2009).

Decadal trends in ocean–climatic observations within the area show a generalized sea surface warming, shallowing of the summer coastal thermocline depth, weakening of the upwelling intensity during most of the year along the western Iberian coast, and intensification in upwelling intensity during peak summer along the southwestern Iberian coast (Lemos and Sansó, 2006; Álvarez *et al.*, 2008; Relvas *et al.*, 2009; Pardo *et al.*, 2011; Santos *et al.*, 2011).

Time-series data on phytoplankton and microbes are available for nine sites distributed along the continental shelf of this region, with reasonably good coverage of the southern Bay of Biscay. Available sites include two confined coastal ecosystems at the land–ocean interface, the Nervión lower estuary and the Guadiana upper estuary. For these two sites, in addition to general oceanographic drivers described above, local conditions (e.g. rainfall and river-flow patterns) and anthropogenic pressures may cause departures from what is expected in coastal, temperate ecosystems (Cloern and Jassby, 2010). Information available for all sites includes abiotic variables and phytoplankton, usually discriminated into functional groups (e.g. taxa or size classes), whereas data on heterotrophic bacterioplankton are limited to two sites.

Seasonal cycles of planktonic microbes globally reflect the stratification–destratification cycle, further enhanced by coastal upwelling events and riverine inputs. There is no common seasonal cycle of phytoplankton using surface chlorophyll concentration within the region. For instance, unimodal and bimodal annual cycles are found, likely related to site-specific differences in inorganic nutrient loading or light limitation. Sites less affected by coastal upwelling (e.g. Men er Roue, AZTI Station D2, and the lower Nervión estuary) show a classic bimodal cycle, with maxima around early spring and mid-autumn. When nutrients become limiting in the upper layers owing to strong summer thermal stratification, surface chlorophyll concentrations decrease in these locations to values < 0.2 µg l^{-1}. By contrast, if inorganic nutrients are available year-round, as usually found in most coastal areas and upper estuarine sites, or under frequent and strong upwelling episodes, phytoplankton tend to reach relatively high values (i.e. >1 µg l^{-1}) also during summer, as found, for instance, off the city of A Coruña (Bode *et al.*, 2011b) and at Ouest Loscolo

and Le Cornard. Regardless of the seasonal pattern, chlorophyll peaks do not usually exceed 3 µg l^{-1} at any of the exposed coastal sites included here. The relative importance of the autumn bloom in bimodal annual patterns also varies, from similar or even greater in extent than the spring one to a clearly secondary position. Light limitation becomes important in the turbid Guadiana upper estuary during most of the year, in part explaining phytoplankton unimodal annual cycles.

Most of the sites lack significant interannual trends in phytoplankton biomass, although a significant increase was found in the upper layer (0–50 m) of the Basque coast (eastern Cantabrian Sea) for the 1986–2010 period. In the Guadiana upper estuary, a significant declining trend in phytoplankton abundance for the 1996–2010 period is apparently linked to the effects of increased river damming (Barbosa *et al.*, 2010). The overall lack of consistency in phytoplankton interannual trends across sites could be explained by the limited extension of available time-series (< 12 years at most sites) and/or site-specific differences at the level of long-term variability in environmental determinants, phytoplankton physiology, and mortality. Indeed, over the past two decades, primary production has increased significantly at A Coruña, but apparently decreased in the central Cantabrian Sea (Bode *et al.*, 2011b). Nitrate and phosphate concentrations, key phytoplankton resources, show a significant increasing trend only at the Gijón/Xixón site.

Contributions of larger (nano- and microplankton) phytoplankton groups also differ temporally and spatially. Diatoms tend to show variable annual patterns, ranging between bimodal cycles coincident with those of chlorophyll (e.g. Gijón/Xixón) to unimodal cycles with late spring–summer maxima (e.g. Nervión and Guadiana estuaries). As expected, generally single annual dinoflagellate maxima are usually detected 1–2 months after diatom blooms. Although most of the available time-series are too short to depict significant trends, increases in diatom abundance were significant at the Le Cornard and Ouest Loscolo sites on the French coast. Also significant or close to significant, but opposing, tendencies in diatom abundance over the last decade were detected in the Nervión and Guadiana estuaries, increasing and decreasing, respectively. In the latter site, the diatom decline was concomitant with significant reductions in other phytoplanktonic groups, an association also seen at several of the French coast sites (sharing either increasing or decreasing trends), whereas the diatom increasing tendency was apparently contrary to that of dinoflagellates at Gijón/Xixón. Significant increases in dinoflagellate abundance are detected in the A Coruña and Le Cornard time-series.

Picoplankton in the area is strongly linked to oceanographic features such as the IPC or frontal areas (Calvo-Díaz *et al.*, 2004). Cyanobacteria as ecologically relevant members of this class show distinct seasonal cycles. Maximum abundance (up to 10^5 cells ml^{-1}) is usually found in summer. In the southern Bay of Biscay, highest annual abundance of *Synechococcus* during summer–autumn is accompanied by generally lesser abundance of *Prochlorococcus*, more abundant in open-ocean waters (Calvo-Díaz and Morán, 2006). One striking feature of the latter is its complete absence from the French continental shelf, whereas in the rest of the region, it is only detected for half of the year (roughly September–February). The construction of a river dam in the Guadiana estuary promoted a long-term decline in cyanobacterial abundance, probably owing to augmented summer river-flow and increased retention of water and cyanobacteria benthic life stages behind the dam (Barbosa *et al.*, 2010).

Heterotrophic bacterioplankton abundance ranges from 0.2 to 2 × 10^6 cells ml^{-1} in the two sites with available data. A Coruña and Gijón/Xixón show, however, clear differences in seasonal patterns, likely because of dissimilarities in phytoplankton annual cycles and trophic conditions. A sustained bacterial annual maximum around summer is found at the former site, whereas this period of the year coincides with minimum abundance at the latter (Morán *et al.*, 2011). Opposite tendencies in bacterial biomass for the period 2002–2010, increasing at Gijón/Xixón and decreasing at A Coruña are not significant, but suggest that climate-related changes in the region may be strongly dependent on local conditions.

8.1 Bay of Biscay REPHY sites (Sites 46-49)

Dominique Soudant (primary contact), Myriam Perrière Rumèbe, and Danièle Maurer

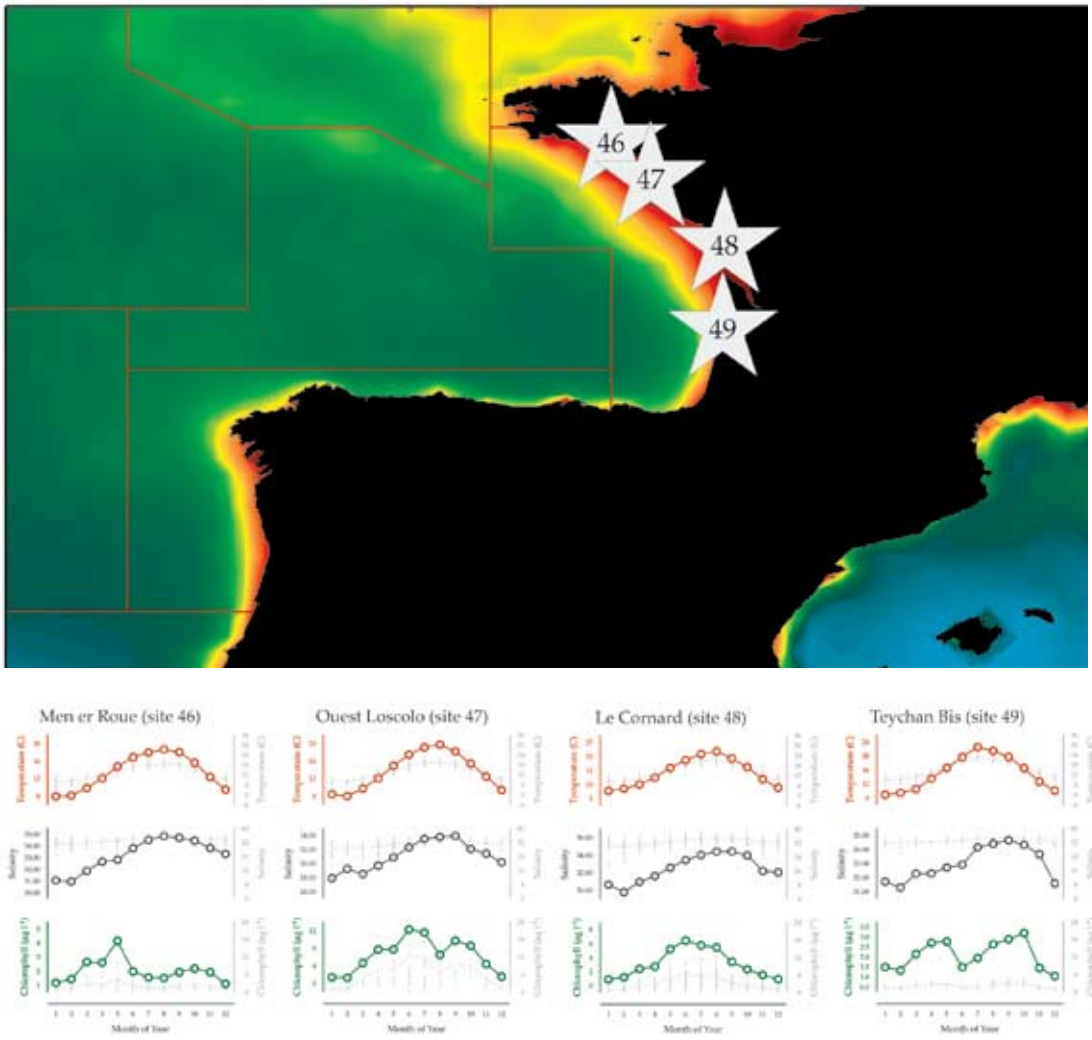


Figure 8.1.1
Locations of the REPHY Bay of Biscay plankton monitoring areas (Sites 46–49), plotted on a map of average chlorophyll concentration, and their corresponding environmental summary plots (see Section 2.2.1).

The French Phytoplankton and Phycotoxin Monitoring Network (REPHY) was set up in 1984 with three objectives: to enhance knowledge of phytoplankton communities, to safeguard public health, and to protect the marine environment (Belin, 1998). Phytoplankton along the French coast has been sampled up to twice a month since 1987 at twelve coastal laboratories. The French coast is divided into a hierarchy of sites and subsites common to three regional networks: the English Channel, the Bay of Biscay, and the Mediterranean Sea. Men er Roue, Ouest Loscolo, Le Cornard, and Teychan Bis are four REPHY sites in the Bay of Biscay. These sites are all shallow, meso- to macrotidal, with differing wave exposure from sheltered in Teychan Bis to moderately exposed at Ouest Loscolo and Le Cornard.

From 1987 onwards, the basic environmental variables salinity, temperature, and turbidity are measured together with phytoplankton composition and abundance. Variables such as inorganic nutrient concentrations chlorophyll *a*, pheopigments, and oxygen were included in the time-series of most of the sites later in different years.

Seasonal and interannual trends (Figure 8.1.2–8.1.5)

Seasonal cycles of chlorophyll as a measure of total phytoplankton biomass are bimodal at Men er Roue (Figure 8.1.2), Ouest Loscolo (Figure 8.1.3), and Teychan Bis (Figure 8.1.5), with maxima generally found in May/June and September/October, which, at the latter site, are even of greater magnitude than the spring peak, and unimodal

at Le Cornard (Figure 8.1.4), with an annual maximum in June. Although dinoflagellates at the sites usually exhibited unimodal cycles, peaking around August, the Teychan Bis site was bimodal, with a strong May peak followed by a weaker August increase. Diatoms at Men er Roue, Ouest Loscolo, and Le Cornard were bimodal, with peaks in May/June and September/October in accordance with chlorophyll cycles. Diatoms at Le Cornard were unimodal, with a strong peak in March and a slow, steady decrease onwards. Dominant diatom species common to all sites include *Skeletonema costatum* and *Leptocylindrus minimus*, with *L. danicus* important at Ouest Loscolo and Teychan Bis. *Asterionellopsis glacialis* may be frequent in blooms at Teychan Bis year-round. *Pseudo-nitzschia* sp. blooms appeared more frequently in the last two years. Dinoflagellates include several species of *Prorocentrum* and *Protoperidinium. Lepidodium chlorophorum* may form summer blooms locally.

Long-term trends for *in situ* temperature, salinity, and chlorophyll are not significant at any of the Bay of Biscay REPHY sites. However, some significant tendencies have been identified in the large phytoplankton groups. Total diatom abundance has increased significantly at the Ouest Loscolo ($p< 0.05$) and Le Cornard ($p< 0.01$) sites. Total dinoflagellate abundance has also increased at both sites, but only Le Cornard's trend was significant ($p< 0.05$). At the Men er Roue and Teychan Bis sites, diatoms and dinoflagellate totals were both decreasing (but without statistical significance). The diatoms:diatoms+dinoflagellates ratio was decreasing at the Teychan Bis site ($p< 0.05$) and at the Ouest Loscolo site (non-significant).

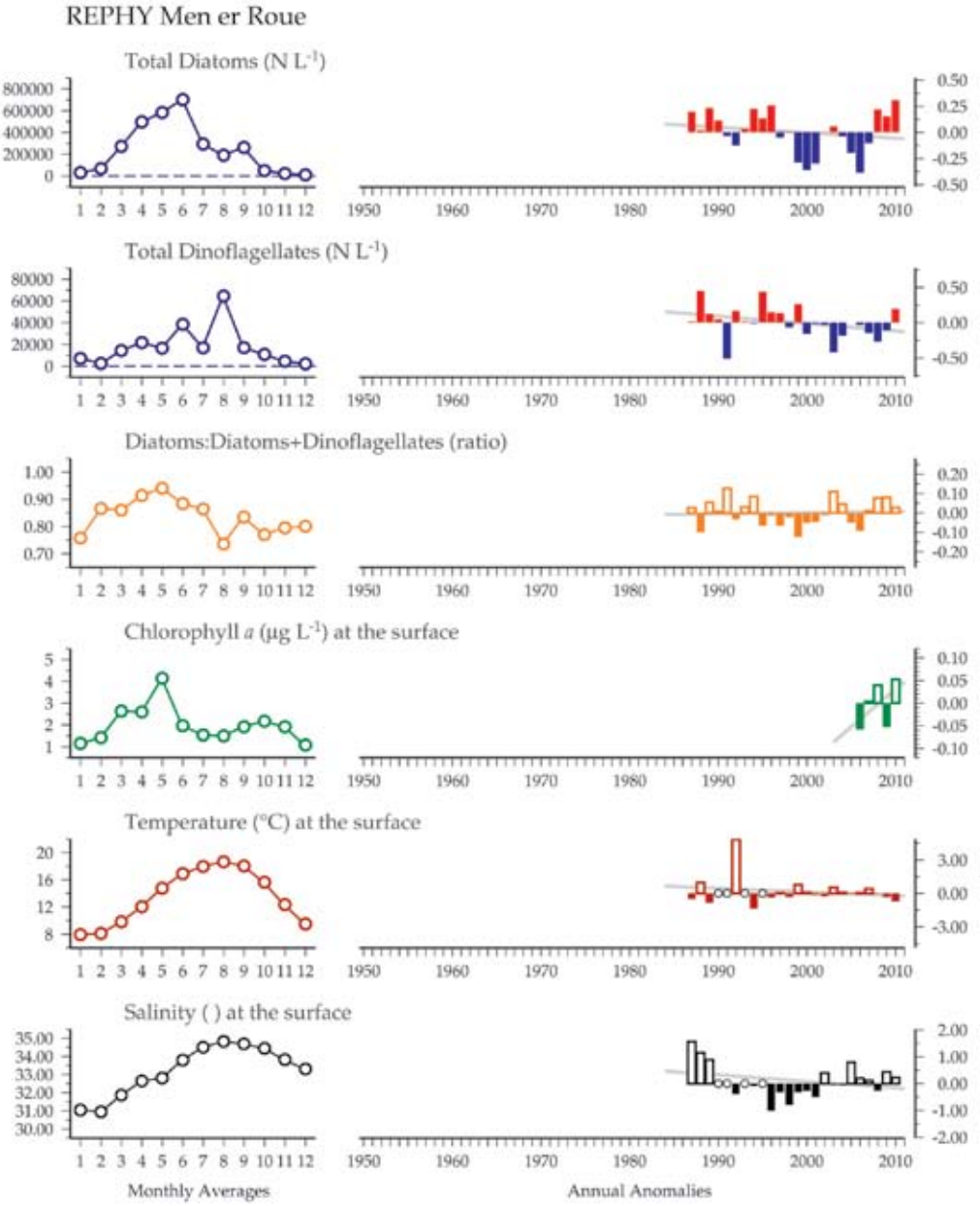


Figure 8.1.2
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the Men er Roue plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.

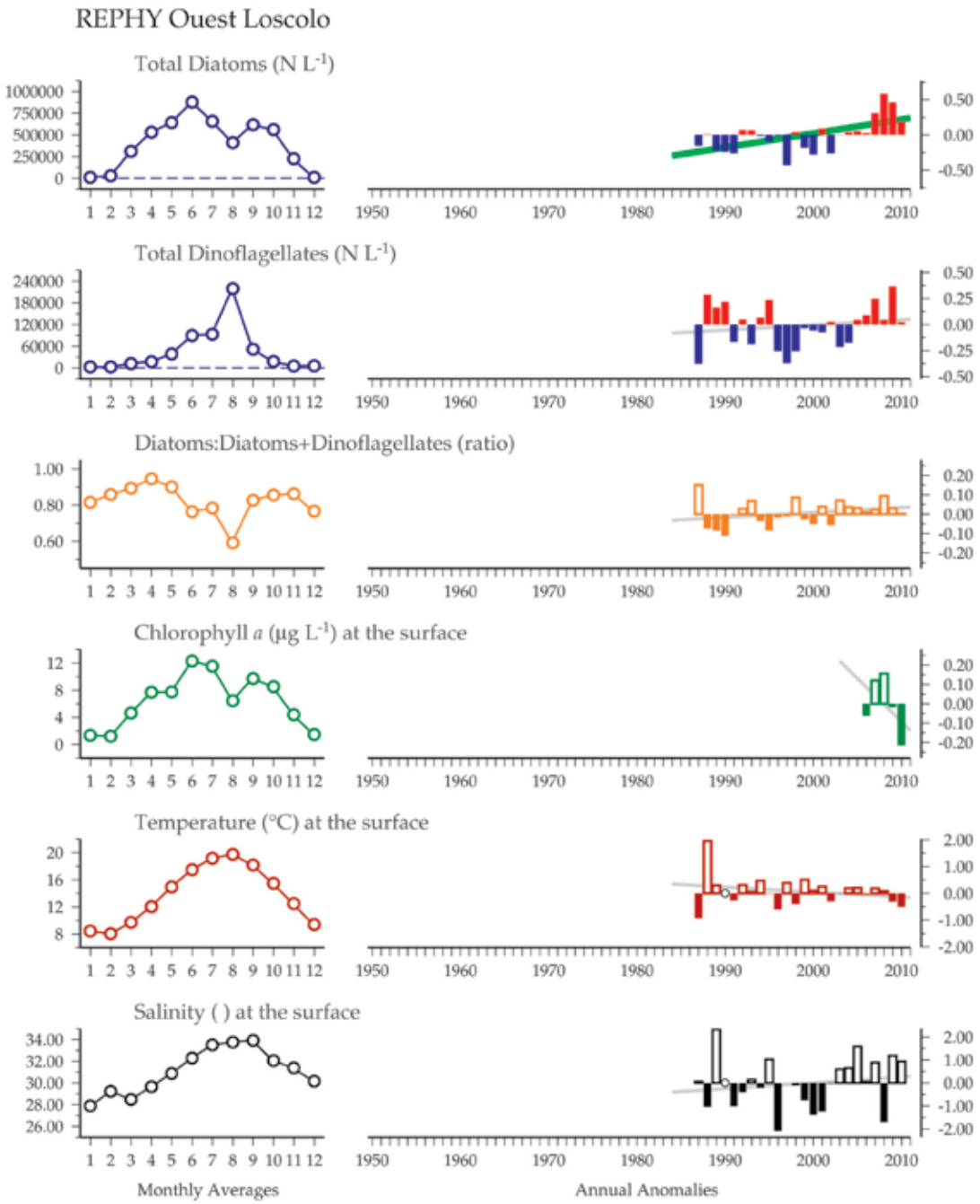


Figure 8.1.3
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the Ouest Loscolo plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.

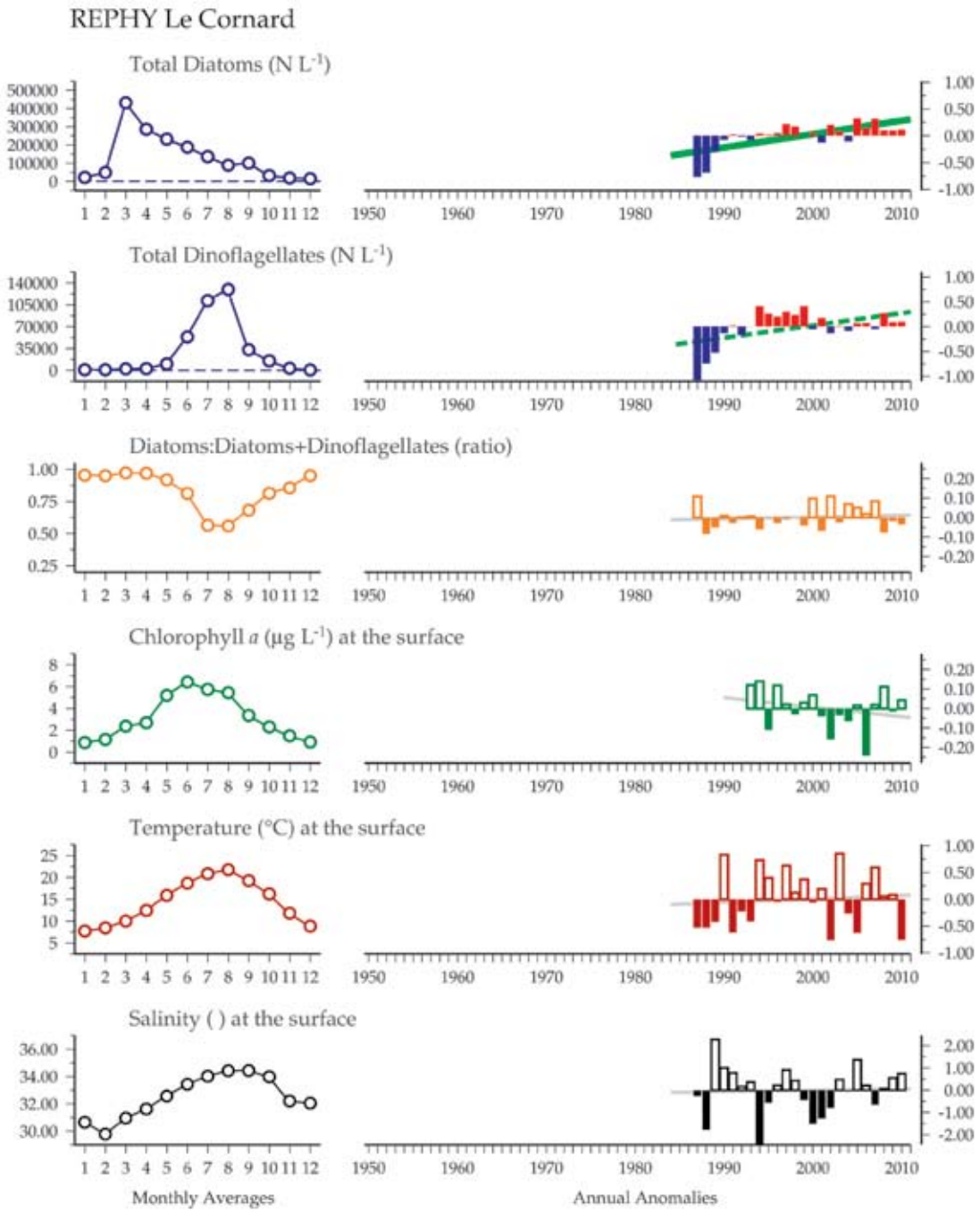


Figure 8.1.4
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the Le Cornard plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.

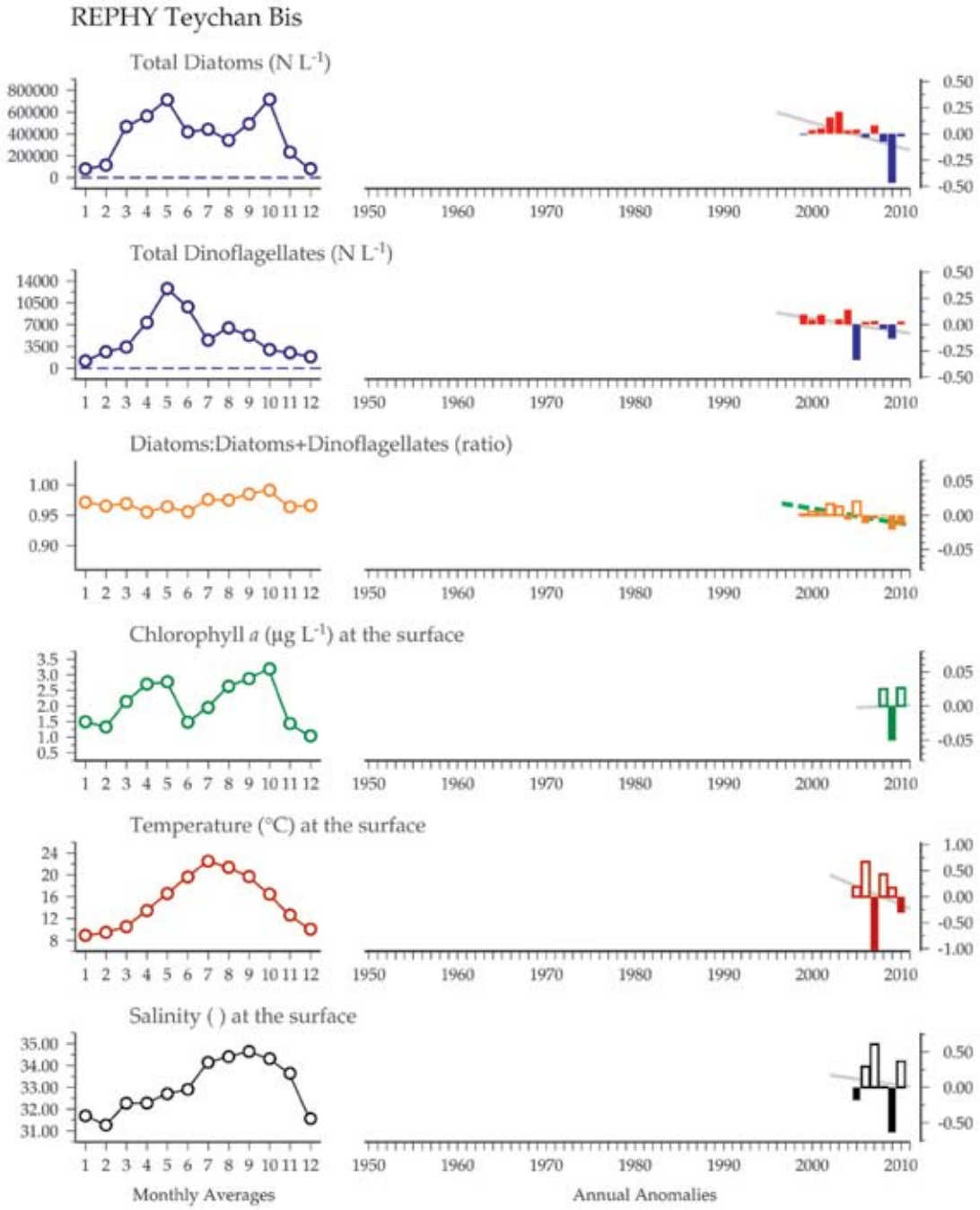


Figure 8.1.5
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the Teychan Bis plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.

8.2 AZTI Station D2 (Site 50)

Marta Revilla, Ángel Borja, Almudena Fontán, and Victoriano Valencia

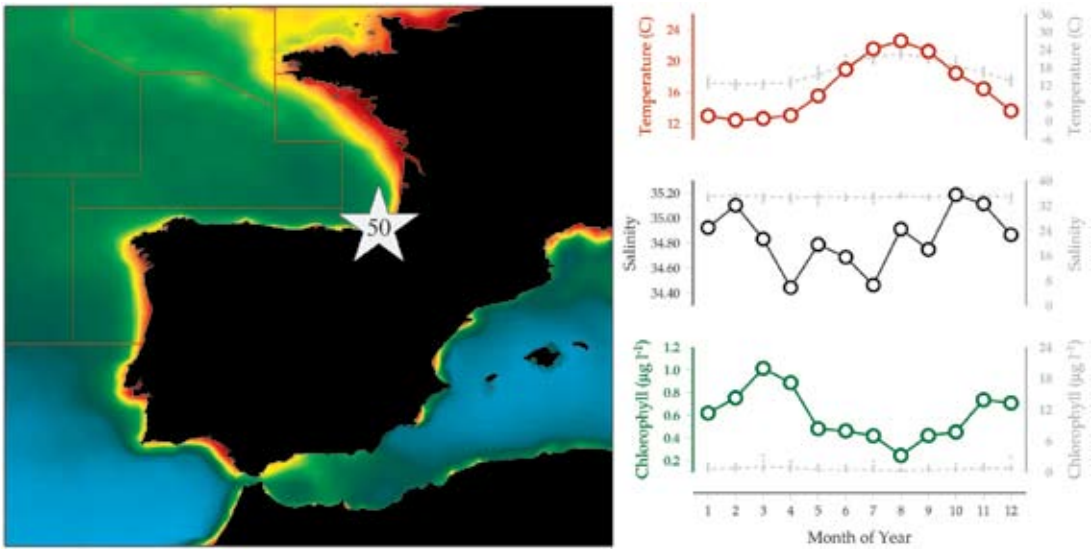


Figure 8.2.1
Location of the AZTI Station D2 plankton monitoring area (Site 50), plotted on a map of average chlorophyll concentration, and its corresponding environmental summary plot (see Section 2.2.1).

Along the Basque coast (southeastern Bay of Biscay), the longest series on phytoplankton biomass (chlorophyll *a* concentration) has been obtained at station D2 (Site 50, 43°27'N 01°55'W). This station is located 13.1 km offshore and at a water depth of 110 m. Data are collected since 1986 by AZTI-Tecnalia, within the project "Variaciones", funded by the Department of Environment, Territorial Planning, Agriculture and Fisheries of the Basque Government. Surveyed months and total number of surveys per year are variable (3–12). On average, eight surveys per year are conducted. Details on sampling and analytical methods are available in Revilla *et al.* (2010). Generally, CTD continuous vertical profiles have been obtained. Here, long-term series on chlorophyll, salinity, and temperature are presented.

Additionally, phytoplankton abundance and composition, together with general environmental conditions (e.g. oxygen, nutrients, and optical properties) are shown from 2002 to 2010. This is part of the Littoral Water Quality Monitoring and Control Network of the Basque Country, conducted by AZTI-Tecnalia for the Basque Water Agency (URA). For this purpose, the station D2 is sampled every three months from February to November. Water samples for phytoplankton analyses (Utermöhl, 1958) are collected at the surface with a clean bucket and immediately fixed with glutaraldehyde. Field and laboratory methods are described in detail by Garmendia *et al.* (2011).

The offshore station D2 is considered to be unaffected by anthropogenic influence, owing to its distance from the main pollution sources on land. The main fertilization factor for the surface waters of this area is the continental run-off coming from the rivers around the southeastern Bay of Biscay (Valencia and Franco, 2004; Díaz *et al.*, 2007; Ferrer *et al.*, 2009). Surface salinity is 34.8 ± 0.67 (average ± standard deviation) from 1986 to 2008 (n = 174), which indicates a low average freshwater content (2.3%).

Seasonal and interannual trends (Figure 8.2.2)

At station D2, sea surface temperature (SST) presents a distinct seasonal cycle, and chlorophyll in surface waters (0–1 m) is inversely correlated to SST. The cold season can be defined as November–April, with monthly averaged SST ranging from 12.5 to 16.5°C and surface chlorophyll ranging from 0.6 to 1.0 µg l^{-1}. The warm season can be defined as May–October (15.6–22.7°C, monthly averaged SST). During the warm months, the mean chlorophyll concentration is below 0.5 µg l^{-1} in surface waters.

The surface waters at D2 station have warmed up over the past 20–30 years ($1.4 \pm 1.1 \times 10^{-2}$ °C $year^{-1}$). The change could be faster for inshore waters, at around 2.4×10^{-2} °C $year^{-1}$ (González *et al.*, 2008; Revilla *et al.*, 2009). Warming patterns have also been described for other neighbouring areas (e.g. Goikoetxea *et al.*, 2009; Bode *et al.*, 2011b). In the

southern Bay of Biscay, Llope *et al.* (2007) linked the recent warming of the ocean surface with increasing stratification and weaker/shallower winter mixing, which could reduce nutrient inputs and cause stoichiometric changes. In this scenario, it has been hypothesized that primary production will be progressively lower and phytoplankton assemblages will change (Llope *et al.*, 2007).

At station D2, Revilla *et al.* (2010) found a slight decrease in the surface chlorophyll concentration between 1986 and 2008 ($-3.8 \pm 4.7 \times 10^{-3}$ µg l^{-1} $year^{-1}$). Further studies demonstrated that the photic-layer-averaged chlorophyll (0–50 m) had followed an opposite, increasing trend ($6.2 \pm 2.5 \times 10^{-3}$ µg l^{-1} $year^{-1}$). In addition, the location of the chlorophyll subsurface maximum could have moved progressively deeper in the water column, from 10 m at the beginning of the series to 30 m in the last decade. These trends could be related to climatic factors, such as the East Atlantic (EA) pattern (ICES, 2011).

The analysis of the annual anomalies at station D2 demonstrates a significant increase ($p < 0.05$) in the photic-layer-averaged chlorophyll over the 1986–2010 period. *In situ* surface temperatures taken immediately at the site had an increasing (but non-significant) trend, whereas HadISST date-matched data from the larger region did present a significant increasing trend ($p < 0.05$). These findings support previous results in the area that were obtained by other methods for trend analyses. Those were non-parametric methods based on seasonal variability removal and regression analysis, or KZA filter (Revilla *et al.*, 2010; ICES, 2011).

Decadal variations in climatic conditions have been described in this geographical area, with an increase in the positive phase of the EA occurring since 1997 (Borja *et al.*, 2008; Goikoetxea *et al.*, 2009). In the northern part of the Iberian Peninsula, the EA pattern is the most important variation pattern explaining temperature variability. In particular, in the southeastern Bay of Biscay, a positive EA involves warmer and drier winters, as well as stronger downwelling, caused by the southwesterly winds. At a larger scale, the positive phase of the EA is associated with above-average precipitation over northern Europe and Scandinavia, and with below-average precipitation across southern Europe (deCastro *et al.*, 2008).

At station D2, the oceano-meteorological variability coupled to the EA pattern could explain the decrease in phytoplankton biomass in surface waters and its increase in deeper waters in response to stronger downwelling and reduced cloudiness (ICES, 2011). Nevertheless, in other areas of the north Iberian Peninsula, the response of phytoplankton to climate variability could be different in relation to the relative influence of other hydrographic factors (e.g. upwelling activity and run-off) at the local scale (Bode *et al.*, 2011b).

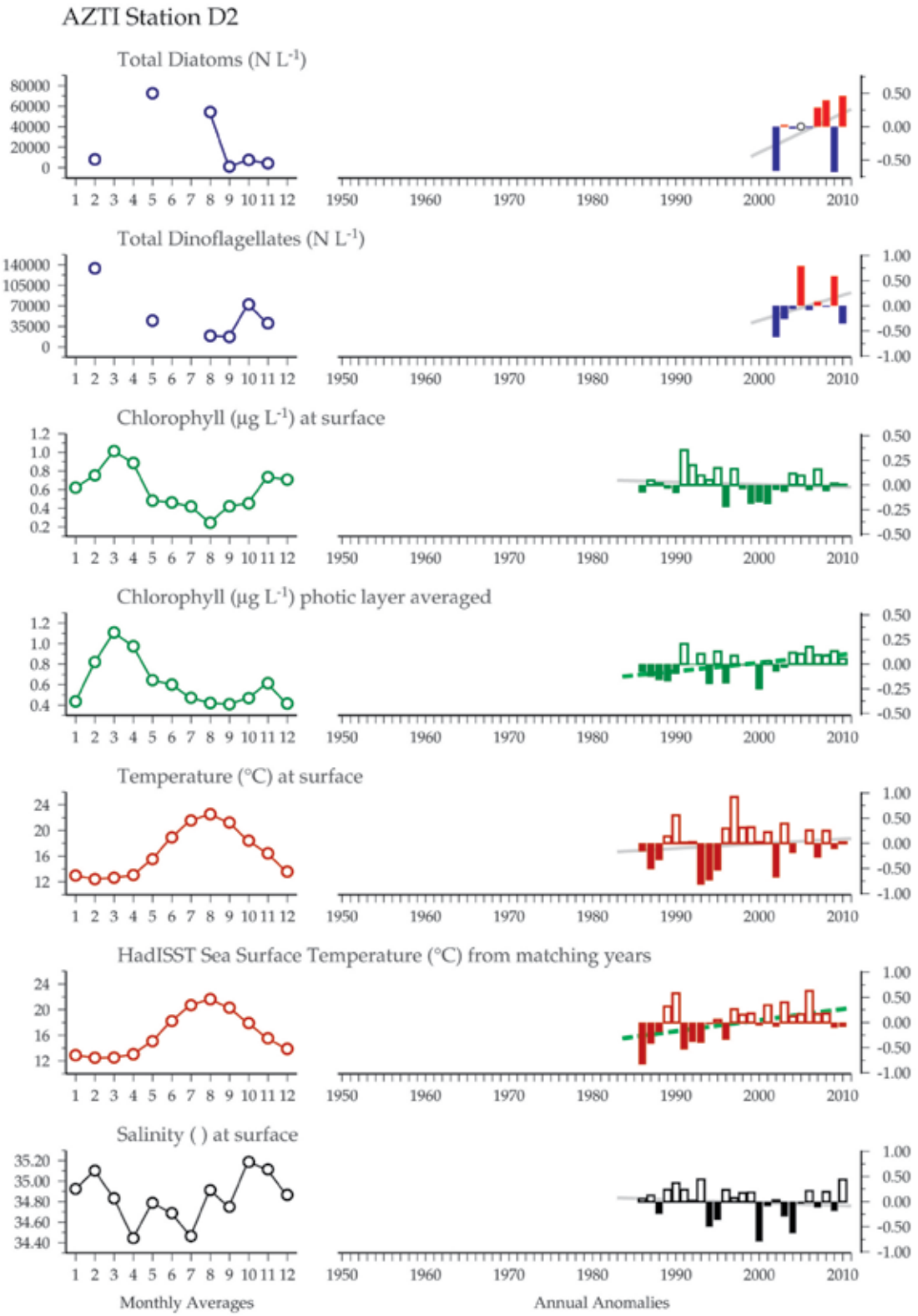


Figure 8.2.2
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the AZTI Station D2 plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.

8.3 Nervión River estuary (Site 51)

Emma Orive, Javier Franco, Aitor Laza-Martínez, Sergio Seoane, and Alejandro de la Sota

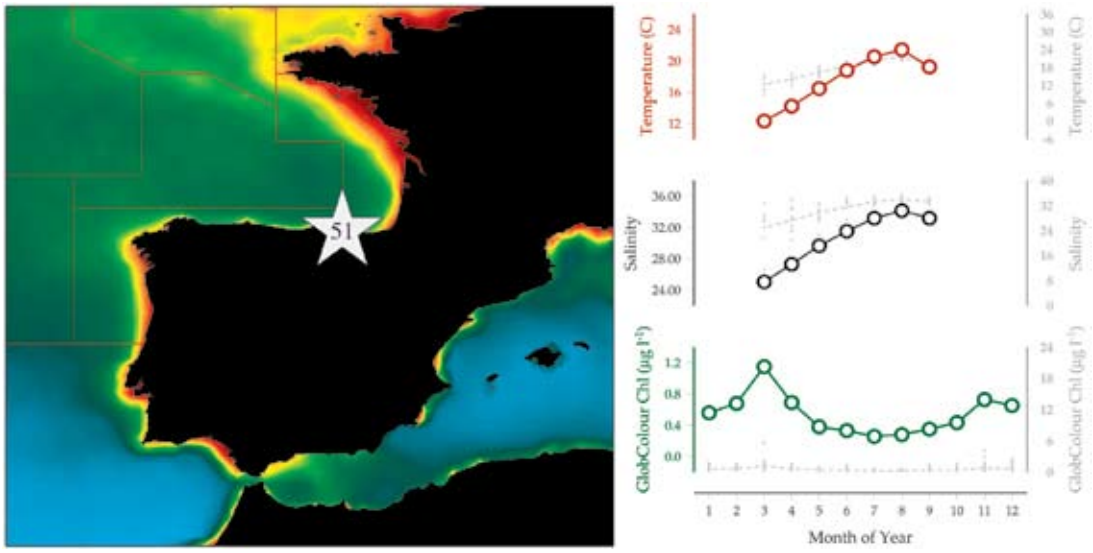


Figure 8.3.1
Location of the Nervión River Estuary plankton monitoring area (Site 51), plotted on a map of average chlorophyll concentration, and its corresponding environmental summary plot (see Section 2.2.1).

The Nervión River estuary (also named Nervión–Ibaizabal estuary) has been monitored for phytoplankton taxonomic composition and abundance since 2000. The Nervión catchment is densely populated, and this study forms part of a broader monitoring programme performed to follow the water quality evolution after the implementation of a sewerage scheme in the area (García-Barcina *et al.*, 2006). Phytoplankton samples are taken aproximately monthly from March to September at eight stations (1–8, from the outer to the inner estuary) located along the longitudinal axis. Samples are analysed for cell identification and counted with the Utermöhl method; in addition, individuals from selected genera among those forming blooms or known to contain harmful species are isolated from live samples and grown in cultures for a more detailed morphological and genetic characterization. Stations 1 and 2 are located in the outer estuary (Abra of Bilbao), which, with a width of ca. 3 km and a maximum depth of ca. 30 m, contains most of the estuarine water. This estuarine area is dominated by marine water during most of the monitoring period and phytoplankton species are mainly of marine origin.

Seasonal and interannual trends (Figure 8.3.2)

The apparent increase in total diatoms and flagellates from 2000 to 2010 can be attributed to the improvement in water quality during this period, and is likewise evident in the diatoms:diatoms+dinoflagellates ratio. Dinoflagellates are relatively scarce in the estuary, as determined by signature pigments from estuarine phytoplankton (Seoane *et al.*, 2005). Fucoxanthin is usually the dominant pigment in the total phytoplankton. Haptophytes are almost always present in the outer estuary (Seoane *et al.*, 2009), and blooms of *Apedinella spinifera* and *Heterosigma akashiwo* have been observed. Diatoms seem to constitute the bulk of chlorophyll *a* among fucoxanthin-containing microalgae. Alloxanthin is also relatively abundant, in agreement with the abundance of cryptophytes, which form frequent blooms both in the outer and inner estuary (Laza-Martinez, 2012). However, in the picoplankton, chlorophyll *b* is almost always the dominant pigment (Seoane *et al.*, 2006). FISH analysis (unpublished data) revealed the dominance of prasinophyceans in this size fraction.

Cell density can be high in spring in the outer estuary because of the spring diatom bloom in adjacent coastal waters. During this period, which can be very short and start at different times each year, the outer estuary is dominated by large marine diatoms responsible for the annual maxima of chlorophyll *a*. However, the maximum abundance for most phytoplankton classes, in terms of cell numbers, is generally registered in summer. The size of the cells, generally larger in spring, can account for the absence of a clear relationship between chlorophyll *a* values and the number of cells. Summer maxima in cell numbers can be partially attributed to the

export to the outer estuary of eurihaline taxa that reach bloom proportions in the inner and middle estuary. In summer, these estuarine segments are a source of small centric diatoms and flagellated forms, belonging to Chlorophyta, Cryptophyta, and Haptophyta, to the outer estuary (Seoane *et al.*, 2006; Laza-Martinez *et al.*, 2007). In addition to these eurihaline species, the outer estuary contains marine forms which grow better in this area during summer than in the oligotrophic coastal waters (Orive, 1989; Orive *et al.*, 2010).

The shift between flagellates and diatoms in the outer estuary in summer is driven by river run-off after rainy periods and by stability of the water column. The raphidophycean *Heterosigma akashiwo*, which is known to support high irradiance levels without experiencing photoinhibition (Martinez *et al.*, 2010), has been observed in bloom proportions in the outer estuary coinciding with very sunny days and elevated temperatures.

Lately, much attention is being paid to potentially toxic epiphytic dinoflagellates, which can appear in the water column as part of the phytoplankton. In addition to several species of the genera *Coolia* and *Prorocentrum*, toxic forms of the genus *Ostreopsis* have been found in the outer estuary and other localities of the southeastern Bay of Biscay that constitute the upper reported limit for the distribution of this pantropical genus in the Atlantic Ocean (Laza-Martinez *et al.*, 2011).

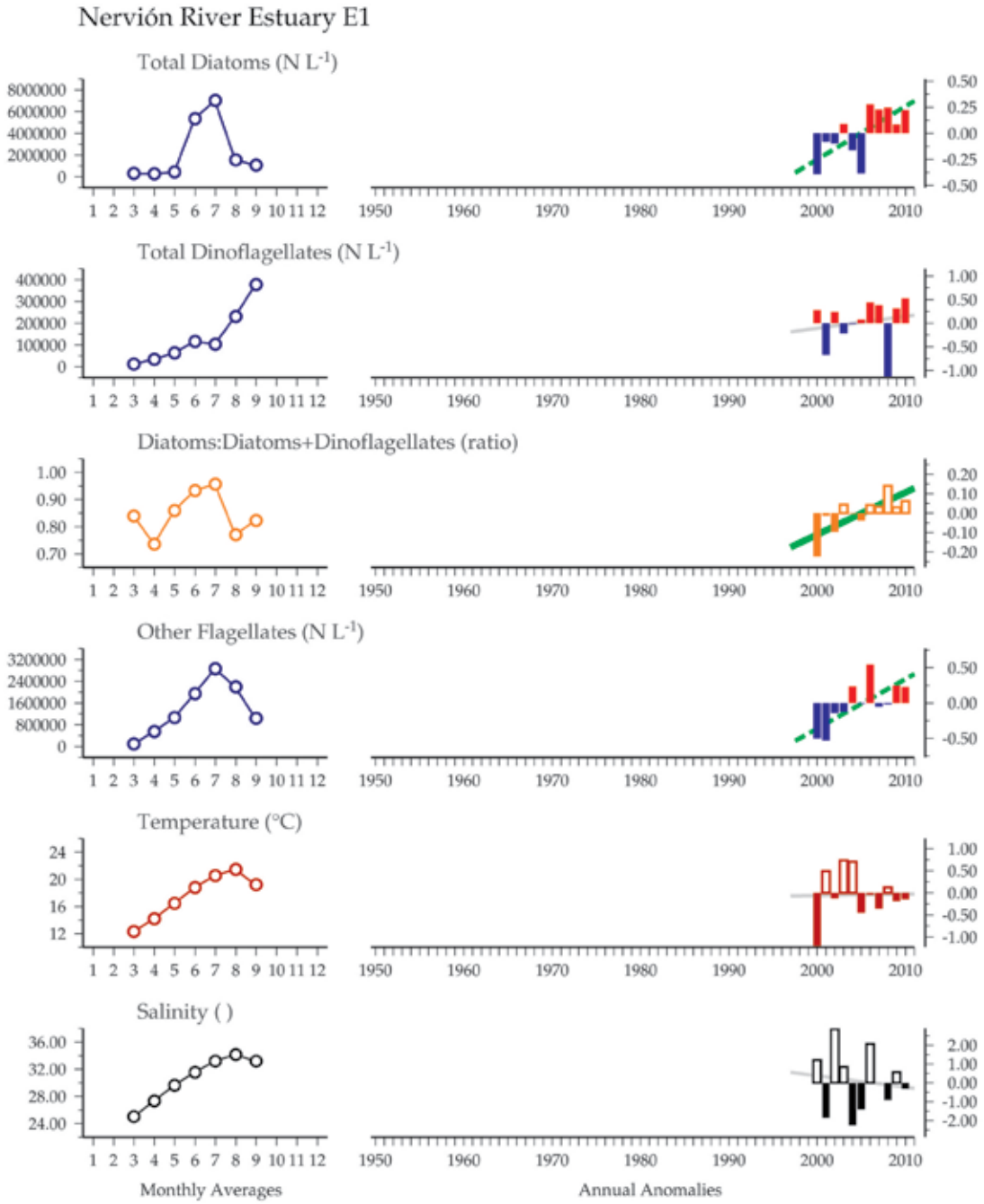


Figure 8.3.2
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the Nervión River estuary plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.

8.4 RADIALES Gijón/Xixón Station 2 (Site 52)

Xosé Anxelu G. Morán and Renate Scharek

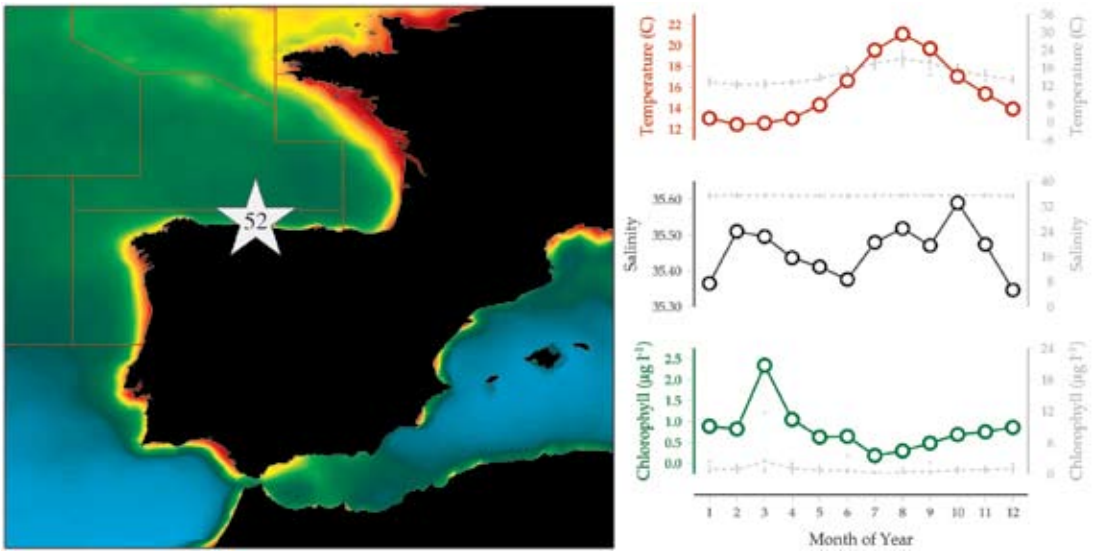


Figure 8.4.1
Location of the RADIALES Gijón/Xixón Station 2 plankton monitoring area (Site 52), plotted on a map of average chlorophyll concentration, and its corresponding environmental summary plot (see Section 2.2.1).

Sampling at the Gijón/Xixón transect started in March 2001, shortly after the opening of the Oceanographic Center of Gijón/Xixón, the latest laboratory of the Spanish Institute of Oceanography (IEO) to be built. Picoplankton began to be collected one year later. The Gijón/Xixón oceanographic time-series is part of the IEO programme RADIALES (http://www.seriestemporales-ieo.com/) and comprises three stations in the central Cantabrian Sea (southern Bay of Biscay) located over the ca. 37 km wide continental shelf off the city of Gijón/Xixón (Asturias, Spain). Only Station 2 data were used for this site summary.

Water samples are collected monthly on board RV "José de Rioja" from Niskin bottles in a rosette sampler attached to a CTD probe. The three stations are sampled for picoplankton at 10 m intervals from the surface down to 50 m, and additionally at 5 m at Station 1, at 75 and 100 m at Station 2, and at 75, 100, and 150 m at Station 3. Larger phytoplankton (nano- and microphytoplankton) is sampled at 0, 30, and 75 m at Station 2. Autotrophic and heterotrophic picoplanktonic groups are distinguished by flow cytometry analysis of 1% paraformaldehyde plus 0.5% glutaraldehyde preserved, ultrafrozen samples. Autotrophic picoplankton groups or picophytoplankton (*Synechococcus* and *Prochlorococcus* cyanobacteria and two groups of picoeukaryotes) are distinguished by red and orange fluorescence and size signals of thawed, unstained samples, whereas heterotrophic bacteria are first dyed with SYTO 13 fluorochrome. Nano- and microphytoplankton are fixed with acetic-acid Lugol´s solution (1% final concentration). Qualitative and quantitative analysis of nano- and microplankton is performed with an inverted microscope using the Utermöhl technique (Utermöhl, 1958). Cells are classified to species or genus level if possible or assigned to higher taxonomic levels divided into size classes.

The site displays the typical oceanographic conditions of a temperate shelf sea, with a well-mixed water column from November through April, broken occasionally by the presence of low-salinity water at surface layers and conspicuous stratification in late spring and summer, with a pycnocline usually developing at 10–20 m depth (Calvo-Díaz and Morán, 2006). Other hydrographic features include the presence of a saline and warm poleward slope current, especially during winter (Pingree and Le Cann, 1990; Álvarez-Salgado *et al.*, 2003) and short-lived, upwelling pulses more frequently found in late summer and early autumn (McClain *et al.*, 1986; Llope *et al.*, 2006). Warming trends have been described for the region for both surface (deCastro *et al.*, 2009) and deeper waters (González-Pola *et al.*, 2005), similar to those described for the whole North Atlantic basin (e.g. Johnson and Gruber, 2007).

Seasonal and interannual trends (Figure 8.4.2)

Total phytoplankton biomass (chlorophyll) demonstrates a unimodal distribution, with the annual maximum associated with the spring bloom in March and April, and minimum values usually recorded in July. As previously described (Calvo-Díaz and Morán, 2006), a marked seasonality in picophytoplankton becomes evident, with late summer–early autumn maxima in abundance (>10^5 cells ml^{-1}) and predominance of cyanobacteria (>80% of total abundance), and minima in early spring (< 10^4 cells ml^{-1}), coincident with very small numbers of *Synechococcus*. One prominent feature is the absence of *Prochlorococcus* for roughly half of the year (March–July), probably due to a combination of low temperatures and high mortality rates in winter, with water-mass advection playing a role in its reappearance in late summer (Calvo-Díaz *et al.*, 2008). The abundance of this cyanobacteria appears related to water temperature, consistent with the predicted increase in picophytoplankton absolute and relative abundance in a warmer North Atlantic (Morán *et al.*, 2010).

Diatom spring blooms occur around April and, in some years, late summer–autumn diatom blooms are also found, which can be as pronounced as or even more pronounced (in terms of cell abundance) than the spring bloom. As expected for an open coastal area, big dinoflagellates (>20 µm) do not form marked blooms. Small dinoflagellates (< 20 µm) formed a bloom in late autumn 2006, and during the spring bloom in April 2007, increased to nearly half of the cell concentration of diatoms. Representative diatom species forming spring or autumn diatom blooms are *Chaetoceros* spp., *Hyalochaete* spp., *Pseudo-nitzschia* spp., *Rhizosolenia setigera*, *Rhizosolenia pungens*, and *Guinardia delicatula*.

Unlike most autotrophs, heterotrophic bacteria (0.2–2.7 × 10^6 cells ml^{-1}) show a clearly bimodal distribution, with peaks in April and October and relative minima in February and July. Highly consistent changes in the relative distribution of the flow cytometric groups of cells with low and high nucleic acid content probably reflect distinct species succession (Morán *et al.*, 2011). Bacterial abundance off Gijón/Xixón has increased since the beginning of the time-series. Although this trend is not significant (grey line in Figure 8.4.2), an analysis decomposing the monthly time-series variance has demonstrated a significant increase in integrated bacterial biomass, with the 2009 annual mean value being 30% higher than that of 2002 (Morán *et al.*, 2011).

By 2012, we will have completed a decade of microbial records in this site. Although it will probably still be too short to draw concluding associations with the observed increases in oceanic temperature, our working hypothesis is that small plankton will become increasingly important in the near future.

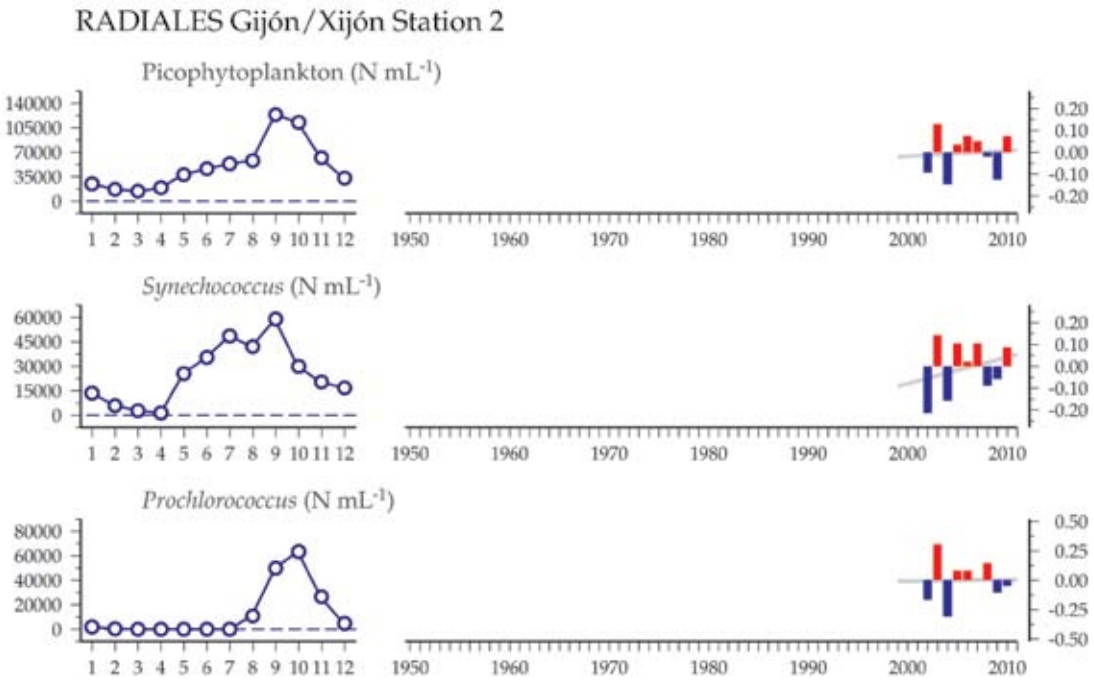
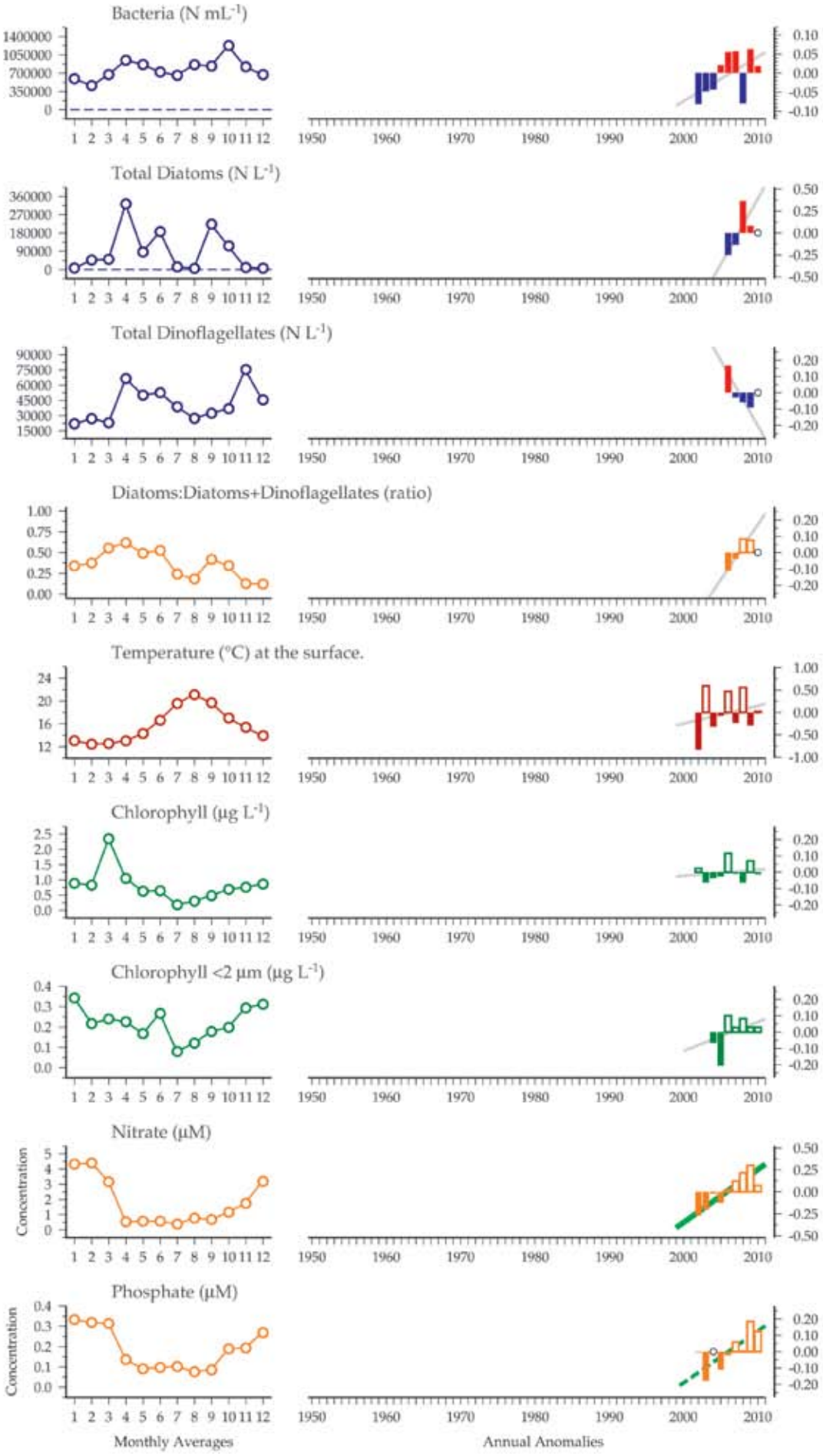


Figure 8.4.2 (continued on facing page)
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the RADIALES Gijón/Xixón Station 2 plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.

Figure 8.4.2 continued.



8.5 RADIALES A Coruña Station 2 (Site 53)

Antonio Bode, Manuel Varela, and Xosé Anxelu G. Morán

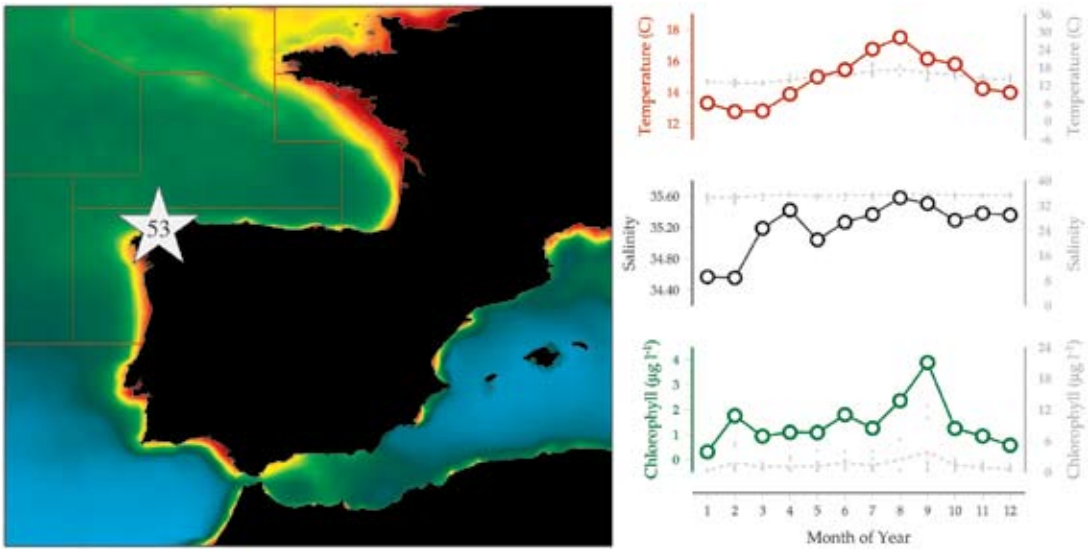


Figure 8.5.1
Location of the RADIALES A Coruña Station 2 plankton monitoring area (Site 53), plotted on a map of average chlorophyll concentration, and its corresponding environmental summary plot (see Section 2.2.1).

The A Coruña section is part of the time-series programme RADIALES (http://www.seriestemporales-ieo.com). Station 2 of the A Coruña section, which was used for this summary, is located off the northwestern Iberian coast at 43°25.3'N 8°26.2'W. Phytoplankton samples were collected at the depth equivalent to irradiance levels 100, 50, 25, 10, and 1% of surface photosynthetic active irradiance (PAR) using Niskin bottles. Sampling (starting in 1989) is made at monthly frequency. CTD casts were made simultaneously with sampling (Sea-Bird rosette from 2000 onwards) or just before bottle deployment (1989–1999) and temperature, salinity, *in situ* chlorophyll fluorescence, and PAR irradiance profiles were recorded. Aliquots of water for large phytoplankton samples were preserved in Lugol's iodine. Starting in 2004, picoplankton was sampled and analysed as described by Calvo-Díaz and Morán (2006). Additional water samples were collected for determination of chlorophyll (GF/F filters, acetonic extracts, fluorometry) and nutrients (nitrate, nitrite, ammonium, phosphate, and silicate by segmented flow analysis). Detailed description of sampling and analytical procedures can be found in Casas *et al.* (1997).

The phytoplankton cycle in this area demonstrates the general characteristics described for the temperate zone (Margalef, 1964; Varela *et al.*, 2001). There is an alternation between a mixing period in winter and a stratification period in summer, with blooms in the transition phases: mixing–stratification (spring blooms) and stratification–mixing (autumn blooms). In this section, as in the whole coastal region off Galicia (northwestern Spain), this classical pattern of seasonal stratification of the water column in temperate regions is masked by upwelling events, especially during summer (e.g. Casas *et al.*, 1997). These upwelling events provide phytoplankton populations with favorable conditions for development well beyond the typical spring bloom of most seas in this latitude. As a consequence of these disturbances of the water column, phytoplankton abundance demonstrates a unimodal distribution, with annual maxima in summer and predominance of diatoms throughout the year. Only during stratification periods, coinciding with a decrease in upwelling intensity, is there a relative increase of dinoflagellates (Margalef *et al.*, 1955; Varela *et al.*, 1996; Casas *et al.*, 1997, 1999).

Seasonal and interannual trends (Figure 8.5.2)

Phytoplankton blooms can be observed from spring to autumn, including summer, when major phytoplankton abundance was also detected. Diatoms always dominate the microphytoplankton community. Several species of the genus *Chaetoceros*, especially *C. socialis* as well as *Leptocylindrus danicus*, *Pseudo-nitzschia pungens*, and *Skeletonema costatum*, are generally dominant during blooms in all seasons. During spring, *Cerataulina pelagica*, *Lauderia annulata*, *Detonula pumila*, and *Guinardia delicatula* are more typical, whereas in autumn,

outbursts of *Asterionellopsis glacialis, P. delicatissima*, and *Thalasssiosira levanderi* were more representative of the diatom community. All of the above-mentioned species can be found in the summer upwelling blooms, although *L. danicus, S. costatum, P. pungens*, and *Nitzschia longissima* attained higher abundance in this season. During winter mixing, the phytoplankton community is characterized by species that are present throughout the year, but in very low densities, as well as resuspended diatoms from the sediment (Casas *et al.*, 1999). The diatom species composition is similar to those found in other Galician coastal areas (Bode *et al.*, 1996; Varela and Prego, 2003; Varela *et al.*, 2005, 2006; Prego *et al.*, 2007).

In contrast to studies conducted in neighboring temperate areas, dinoflagellates never dominated during summer stratification periods, probably because of the very frequent upwelling events, which caused destabilization of the water column. In these stratified periods, a relative increase in dinoflagellate populations was recorded. Small species (< 20 µm) of naked dinoflagellates are the most abundant, with average values around 60 cells ml^{-1}, followed by *Prorocentrum balticum, Katodinium glaucum*, and large (>20 µm) unidentified thecate species exhibiting concentrations lower than 7 cells ml^{-1}. In other periods, the composition is similar to total densities around 50 cells ml^{-1} in autumn and upwelling periods, and ca. 30 in spring and winter. *Heterocapsa niei* is typical of winter mixing. *P. balticum* and *P. minimum* are frequently observed during spring and autumn blooms, whereas *Scrippsiella trochoidea* and *Ceratium lineatum* are more characteristic of summer upwelling. As in the case of diatoms, dinoflagellate species in the A Coruña transect are reported for other coastal areas of Galicia (Figueiras and Niell, 1987; Figueiras and Pazos, 1991; Bode and Varela, 1998; Varela *et al.*, 2005, 2008, 2010).

Picophytoplankton abundance at the surface, ranging over two orders of magnitude (10^3–10^5 cells ml^{-1}), tends to be higher during summer, in which cyanobacteria (mostly *Synechococcus*, with *Prochlorococcus* much less abundant and detected only from September through January) may contribute up to 80% of total cell counts. Similar to both large and small phytoplankton, surface heterotrophic bacteria ($0.1–2.2 \times 10^6$ cells ml^{-1}) show consistent unimodal distributions in A Coruña, with maxima found through spring and summer, and minima in February.

Recent studies of the historical data in the A Coruña section demonstrated a significant decrease in diatom abundance at interannual scales, along with an increase in dinoflagellates (albeit non-significant). These results are consistent with the decreasing upwelling intensity in the area (Bode *et al.*, 2011b; Varela *et al.*, 2012). The series revealed that there is no significant long-term trend in phytoplankton biomass, but primary production increased significantly, despite significant decadal changes in upwelling intensity (Bode *et al.*, 2011b). An increase in the use of remineralized nutrients from organic matter has been proposed to explain the increase in production (Pérez *et al.*, 2010). Additional annual observations are required for demonstrating the apparent trend of decreasing bacterial biomass at A Coruña Station 2.

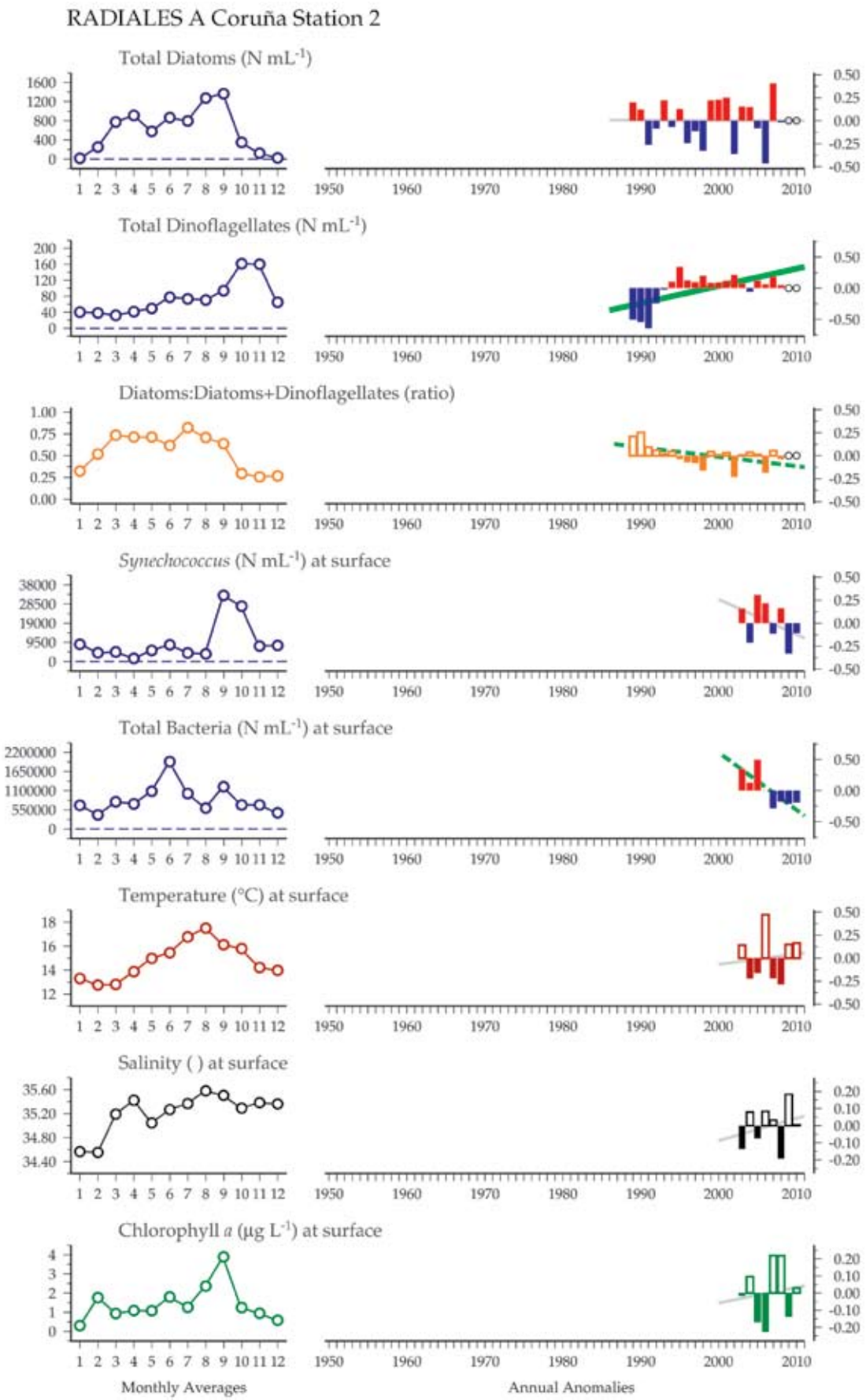


Figure 8.5.2
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the RADIALES A Coruña station 2 plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.

8.6 Guadiana estuary (Site 54)

Ana B. Barbosa, Rita B. Domingues, and Helena M. Galvão

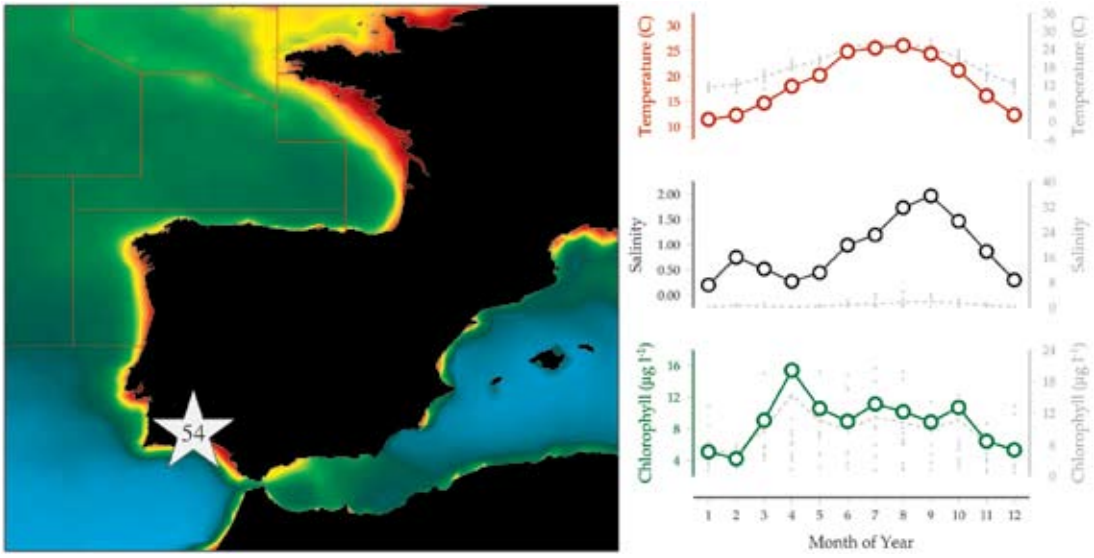


Figure 8.6.1
Location of the Guadiana estuary plankton monitoring area (Site 54), plotted on a map of average chlorophyll concentration, and its corresponding environmental summary plot (see Section 2.2.1).

The Guadiana estuary (GE) is located in southwestern Iberia and is a 22 km^2 mesotidal ecosystem (average depth: 6.5 m), ranging from partially stratified to well-mixed. GE is located in a Mediterranean climate area, classified as highly sensitive to climate change, and is currently considered one of the best preserved and most vulnerable estuaries of the Iberian Peninsula. The Guadiana River watershed is the fourth largest river basin in the Iberian Peninsula and demonstrates a torrential hydrographic regime, with concentrated rainy periods and a prolonged dry season, usually from May to September. Managing water availability under such demanding conditions led to the construction of hundreds of dams, almost 90 of which have a volume capacity over 1 hm^3. Recent construction of the large Alqueva dam, built in the upper estuary in 1999 and completed in 2002, further increased freshwater flow regulation up to 81% (see Barbosa *et al.*, 2010, and references therein).

Phytoplankton monitoring in GE began in 1996 and was motivated by an anticipated increase in cyanobacterial blooms caused by the construction of the Alqueva dam. Physical–chemical variables (e.g. temperature, salinity, intensity of photosynthetic available radiation, light extinction coefficient, dissolved oxygen, concentration of dissolved inorganic macronutrients, particulate suspended matter) and phytoplankton abundance, composition, and biomass were routinely monitored at different stations in the upper, middle, and lower estuary in the framework of projects funded by the Portuguese Foundation of Science and Technology (FCT) and the European Union. Abundance of heterotrophic bacterioplankton and phytoplankton and bacterial production were less frequently determined. Water quality and phytoplankton are also regularly monitored by the Portuguese Water Institute (INAG). Since 2008, water quality and hydrodynamics have been measured by an autonomous instrumented platform positioned at the estuary entrance (Garel *et al.*, 2009).

Data displayed in this site are average monthly values (1996–2010) collected at station "Alcoutim" (37°28'N 7°28'W; Datum WGS84) at subsurface levels (ca. 0.5 m). The station is located ca. 38 km from the Guadiana river mouth within the upper Guadiana estuarine region and has a mean depth of 9 m. Details on sampling and analytical methods are given in Barbosa *et al.* (2010). Samples were usually collected from the Alcoutim pier using a sampling bottle, and sampling frequency was usually monthly during autumn–winter and biweekly during the spring–summer productive period. Physical–chemical variables were analysed using standard methods. Chlorophyll *a* concentration, used as a proxy for phytoplankton biomass, was analysed using a spectrophotometric method, after GF/F sample filtration and acetone extraction. Abundance of picoplankton (< 2 µm) and nanophytoplankton (2–20 µm) was estimated using epifluorescence microscopy, after gluteraldehyde preservation and proflavin staining. Abundance of microphytoplankton (>20 µm) was analysed using

inverted microscopy, after Lugol preservation (see Barbosa *et al.*, 2010 for details).

Seasonal and interannual trends (Figure 8.6.2)

Phytoplankton seasonal patterns, studied before, during, and after the construction of the Alqueva dam, revealed bimodal to unimodal annual cycles, with chlorophyll *a* maxima usually occurring between March and October. Seasonal patterns show an alternation between a persistent light limitation and episodic nutrient limitation (Rocha *et al.*, 2002; Domingues *et al.*, 2005, 2011a,b,c, 2012; Barbosa *et al.*, 2010). Phytoplankton succession, with spring diatom blooms and summer–early fall cyanobacterial blooms, is driven by changes in nutrients, water temperature, and turbulence, clearly demonstrating the role of river flow and climate variability.

Diatom, green algae, cyanobacteria, and total phytoplankton abundance displayed significant interannual declining trends over the period 1996–2010. Light intensity in the mixed layer is a prevalent driver of phytoplankton interannual variability. Yet, increased water retention by the Alqueva dam led to interannual decreases not only in turbidity but also in nutrient inputs, promoting a shift from persistent light limitation towards a more nutrient-limited mode (see Barbosa *et al.*, 2010; Domingues *et al.*, 2012).

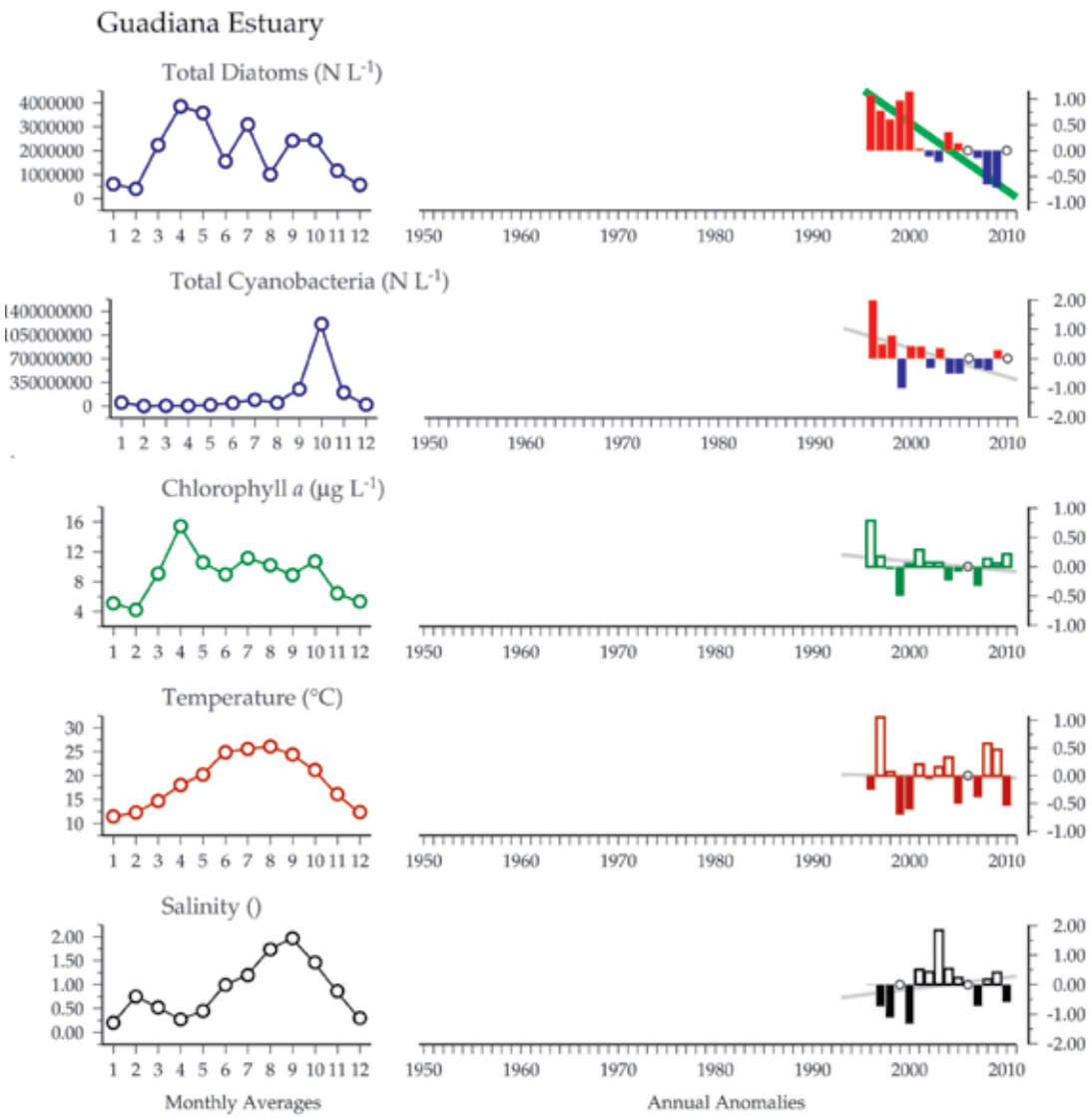
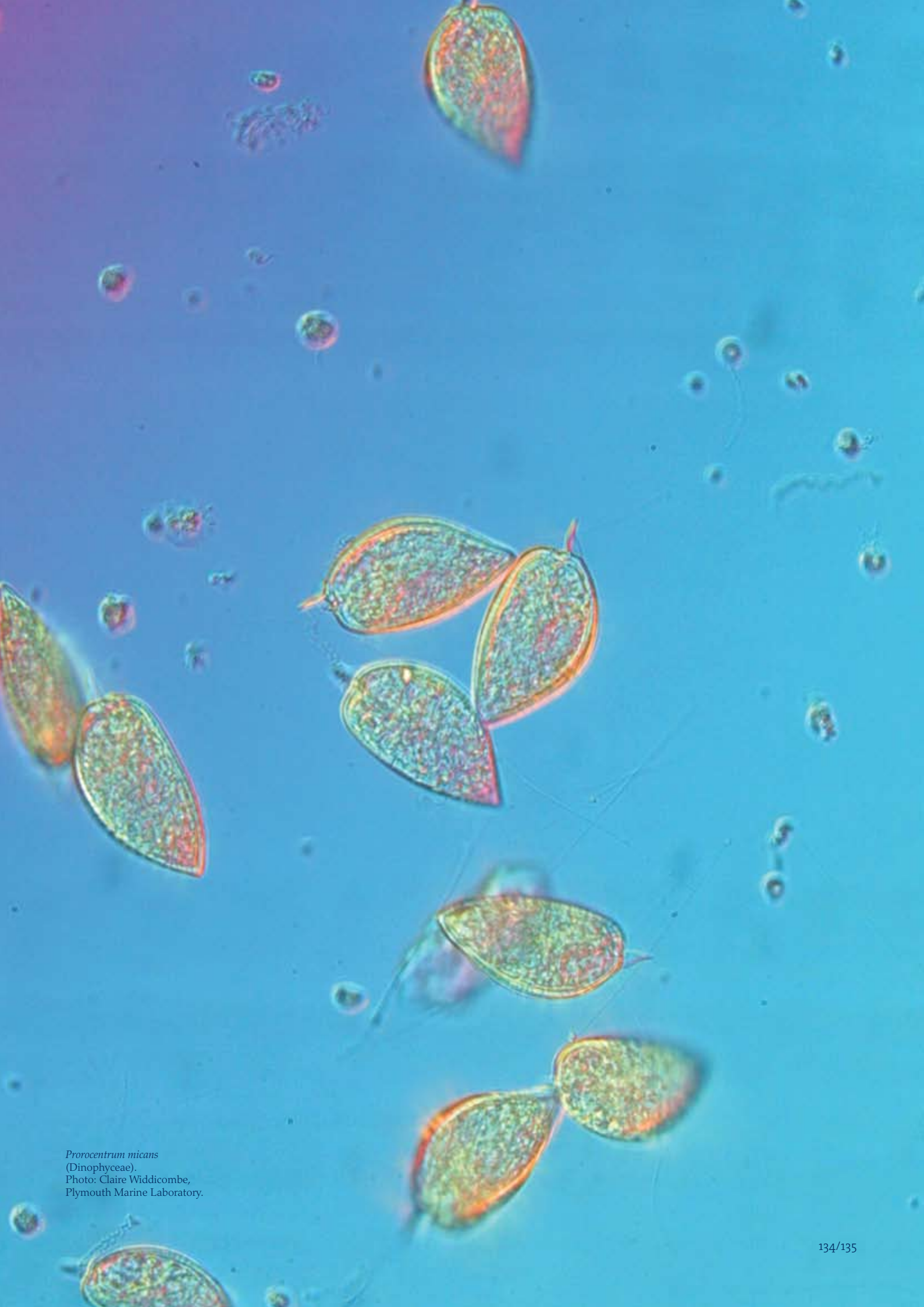


Figure 8.6.2
Multiple-variable comparison plot (see Section 2.2.2) showing the seasonal and interannual properties of select cosampled variables at the Guadiana estuary plankton monitoring site. Additional variables from this site are available online at http://wgpme.net/time-series.



Prorocentrum micans
(Dinophyceae).
Photo: Claire Widdicombe,
Plymouth Marine Laboratory.